REMARKS/ARGUMENTS

Claims 1 to 4 and 6 to 20 are currently pending. Claim 5 was previously canceled and claims 17-20 were previously withdrawn.

No listing of claims is included in this response as there are no claim amendments subsequent to the response filed May 9, 2008.

Interview Summary

Applicants thank Examiner Yang for the courtesy of a telephone interview on November 26, 2008 with its representative, Richard Polley and with Ms. Sally Hemming, Canadian agent for the Applicants. The Eaton et al. reference (*J Biol. Chem.* 277 (24): 21189-21196, 2002) was discussed, and the Examiner requested that the Applicants include detailed remarks in a written response. No agreement was reached with regard to the claims.

Rejections under 35 USC 103

The Examiner rejected claims 1 to 3, 9 and 10 under 35 USC 103 as unpatentable over Nock et al. in view of Eaton et al. The Examiner stated that Nock et al. teaches a method of immobilizing a polypeptide to a surface using mutant inteins and that Eaton et al. teaches that biotinylated cysteine acts as a probe for thiolated proteins, detectable by non-reducing Western blots.

As acknowledged by the Examiner, Nock et al. fails to teach that the ligand is cysteine-biotin. The Examiner now cites Eaton et al. and argues that Eaton et al. provides motivation to use cysteine-biotin to splice the fusion protein of Nock et al.

Applicants respectfully submit that the claims are not obvious having regard to the combination of the Nock and Eaton references, for at least the following reasons.

As previously stated, independent claims 1 and 9 specify the attachment of cysteine-biotin to the C-terminus of the remaining portion of the fusion protein via a peptide bond so that the biotin moiety is attached to the backbone of the fusion protein. As stated in paragraph [0042] of the application and as can be readily seen from Figure 1 of the application, cysteine-biotin has the biotinyl moiety attached to the C-terminus of a single cysteine residue via a peptide bond.

The remaining cited claims 2, 3 and 10 depend directly or indirectly from claim 1 or 9 and thus incorporate these features.

As will be appreciated, a peptide bond is the bond found along the backbone of a peptide or protein chain that connects two amino acids together in the chain. A first amino acid is connected to the next amino acid in the chain by reaction of first amino acid's carboxy group (-COOH) with the amino group ($-NH_2$) of the next amino acid to form an amide linkage (-CONH-; also referred to as a peptide bond) between the amino acids, thus forming a continuous backbone that has an N-terminal free amino group, the central alpha carbon of the first amino acid (referred to as $C_{\alpha 1}$), a peptide bond, $C_{\alpha 2}$, peptide bond, $C_{\alpha 3}$, and so on, terminating in a free carboxy group at the C-terminus (e.g. H_2N-$C_{\alpha 1}$-CONH-$C_{\alpha 2}$-CONH-$C_{\alpha 3}$-CONH-….-$C_{\alpha n}$-COOH, depicting the backbone only). The side chains of the amino groups that define each particular amino acid are attached at each C_α, and thus are pendant from the backbone along the length of the peptide or protein chain. Thus, as required in the present claims, the cysteine-biotin is attached to the newly generated C-terminus of the fusion protein via a peptide bond, resulting in a protein having one additional cysteine residue in the chain, linked into the backbone by a peptide bond, followed by a biotinyl moiety attached to the backbone of the protein.

The presently claimed methods result in attachment of small ligands having only a single amino acid residue and a biotinyl moiety attached to the C-terminus of a protein, thus reducing the potential for disruption of the protein activity when the protein is

immobilized in an array. As well, the small size of the cysteine-biotin ligand and the attachment of the ligand so that the biotin is linked to a protein backbone via a peptide linkage provides a specific, ordered orientation of the protein within the array.

In contrast, Eaton et al. does not describe the use of cysteine-biotin to C-terminally label proteins via a peptide bond, as is required in the present method. Instead, the Eaton reference specifically relates to the labeling of proteins at the thiol group found on the side chains of cysteine residues. That is, the side chain of cysteine has a free thiol (-SH) group that readily reacts with other thiol groups to form disulfide bonds (-SS-). These bonds are easily broken by reducing agents, for example reducing agents having free thiol groups, including DTT (dithiothreitiol). The Eaton reference makes reference to *S*-thiolation of proteins as a regulatory mechanism in response to oxidative stress. The Eaton reference indicates that *S*-thiolation is an oxidative modification of cysteine residues of proteins (page 21189, middle of first column) and that potential mechanisms of protein *S*-thiolation include disulfide exchange with low molecular weight mixed disulfides (page 21189, middle of second column). The Eaton reference also indicates that treatment with DTT abolished detection of *S*-thiolation, indicating that the labeling of proteins with cysteine-biotin is occurring via a disulfide linkage at the thiol sidechain of cysteine. Accordingly, the Eaton reference does not provide any motivation to use cysteine-biotin to C-terminally label proteins via a peptide bond, as required by the present claims, and thus does not render the present claims obvious in view of Nock et al.

Thus, Applicants submit that a skilled person would not, having regard to the combination of Nock et al. and Eaton et al., be motivated or led to use cysteine-biotin to modify Nock et al., and that claims 1 to 3, 9 and 10 are patentable over the combination of Nock et al. and Eaton et al.

The Examiner also rejected claims 4, 6 to 8 and 11 to 16 under 35 USC 103, having regard to the combination of Nock and Eaton, further combined with one or more of previously cited references Duan, Bradley et al., Inoue et al., and Xu et al. None of these

references, alone or in combination, combine with Nock et al. and Eaton et al. to overcome the above-described deficiency of the combination of the Nock and Eaton references.

Thus, none of the references cited under 35 USC 103 can combine with Nock et al. and Eaton et al. to render as obvious the present claims as currently amended. Applicants therefore respectfully request withdrawal of the rejections under 35 USC 103.

In view of the foregoing, it is believed that the application is in condition for allowance. Applicants respectfully request entry of this amendment and allowance of the application.

Respectfully submitted,

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